FINAL SCRIPT: APPROVED FOR FILMING

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Title: Half-Segmental Diaphyseal Bone Defect Model in Rats for Evaluating Bone Substitute Performance in Load-Bearing Regions

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. Filming location: Will the filming need to take place in multiple locations? **No**

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length
Number of Steps: 10
Number of Shots: 20

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

1.1. **Jiayi Wu:** We aim to establish a reproducible femoral defect model to evaluate mechanical and osteogenic performance of load-bearing biomaterials.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

1.2. **Shuhan Liu:** The main challenge is the lack of a standardized, reproducible load-bearing defect model for realistic in vivo mechanical assessment.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

1.3. **Yiyun Chen:** Our protocol enables in vivo mechanical evaluation of implants without fixation, offering a simple and reproducible load-bearing defect model.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Ethical Committee at West China School of Stomatology, Sichuan University

Protocol

NOTE: The main steps of this experiment were performed twice (from Step 2.3.1 through Step 3.4.2). The files from the first round are C9608 to C9631, and those from the second round are C9632 to C9646. You can select the file with the best results from these.

2. Surgical Site Preparation and Femoral Exposure in Rats

Demonstrator: Jiayi Wu

2.1. To begin, place the anesthetized rat in a lateral recumbent position on a sterile surgical bench **[1-TXT]**. Shave the lateral thigh area aligned with the femur projection **[2]** and disinfect the skin using 2 percent iodine tincture followed by 75 percent ethanol **[3]**.

2.1.1. WIDE: Talent positioning the rat on its side on a sterile surgical bench. **TXT: Anesthesia (IP): 100 mg/kg of Ketamine + 10 mg/kg of Xylazine**

2.1.2. Talent shaving the lateral thigh area of the rat.

2.1.3. Talent applying 2 percent iodine tincture, then 75 percent ethanol, to disinfect the skin.

2.2. Drape the surgical site using a sterile fenestrated sheet to maintain asepsis **[1]**.

2.2.1. Talent covering the surgical site with a sterile fenestrated sheet.

2.3. Now, using a straight scissor, make a 2 to 3-centimeter longitudinal skin incision along the lateral aspect of the thigh **[1]** to expose the underlying muscles **[2]**.

2.3.1. Talent making a 2 to 3 centimeter incision along the lateral thigh using a straight scissor. **NOTE**: section 2.3.1 were carried out using different tools: one with scissors and the other with a scalpel, as the scalpel proved far less effective than the scissors.

2.3.2. Shot of the exposed muscles.

2.4. Identify the rectus femoris and vastus lateralis muscles **[1]** and use a straight scissor to carefully separate the muscles along the visible white fascia **[2]**.

2.4.1. Talent pointing to the rectus femoris and vastus lateralis muscles.

2.4.2. Talent separating the muscles along the white fascia using a straight scissor.

2.5. Then, with a disposable sterile scalpel, make a longitudinal incision along the muscle attachments to access the femoral surface **[1]**.

2.5.1. Talent making a longitudinal incision along the muscle attachments using a disposable sterile scalpel.

2.6. Perform blunt dissection using a periosteal elevator to detach the muscle attachments **[1]** and fully expose the mid-diaphysis of the femur **[2]**.

2.6.1. Talent using a periosteal elevator to bluntly dissect.

2.6.2. Shot of the exposed the mid-diaphysis of the femur.

3. Half-Segmental Diaphyseal Bone Defect Creation and Bone Substitute Implantation

3.1. Drill vertically downward at the defect site using the bur until a sudden loss of resistance is felt **[1]**. Enlarge the defect by applying controlled horizontal push-pull motions centered around the initial point of penetration **[2]**. Extend the defect longitudinally along the femoral axis and laterally along the femoral diameter to gradually form a semi-cylindrical defect **[3-TXT]**.

3.1.1. Talent drilling vertically into the femur using the bur.

3.1.2. Talent applying horizontal push-pull motions to widen the defect.

3.1.3. Talent extending the defect in longitudinal and lateral directions to create a semi-cylindrical shape. **TXT: Dimensions of the defect: Length: 4 mm; Radius: 1.5 mm**

3.2. Next, insert the load-bearing 3D printed polymethyl or PMMA implant intended for mechanical performance testing into the defect site, ensuring a snug fit **[1-TXT]**.

3.2.1. Talent placing the 3D-printed polymethyl methacrylate implant into the femoral defect. **TXT: Use non-load-bearing Gelatin methacryloyl hydrogels in other rats for comparison**

3.3. Then, using a needle holder, suture the muscle layers with 4-0 *(4-oh)* monofilament sutures, ensuring there is no excessive tension **[1]**.

3.3.1. Talent suturing the muscle layers with 4-0 monofilament sutures using a needle holder.

3.4. Finally, close the skin with interrupted sutures **[1]** and disinfect the surgical site using 2 percent iodophor solution **[2-TXT]**.

3.4.1. Talent performing interrupted skin sutures.

3.4.2. Talent wiping the closed surgical site with 2 percent iodophor solution. **TXT: Offer post-operative care; Euthanize the animal after 4 weeks for histopathological and biochemical analyses**

Results

4. Results

4.1. At 4 weeks post-operation, micro-computed tomography showed that femurs implanted with Gelatin methacryloyl hydrogel exhibited concave defect areas **[1]** and extensive ectopic ossification within the medullary cavity **[2]**.

4.1.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the depression at the middle of the images in images a1 a2 a3*.

4.1.2. LAB MEDIA: Figure 3A. *Video editor: Highlight images a4 and a5*.

4.2. In contrast, femurs in the 3D printed PMMA group showed restored external morphology with the defect area supported by aligned new bone **[1]**, and minimal ectopic ossification in the medullary cavity **[2]**.

4.2.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the middle region in images b1 b2 b3*.

4.2.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the images b4 and b5*.

4.3. Hematoxylin and eosin staining revealed that the Gelatin methacryloyl group contained abundant fibrous tissue within the defect **[1]**, with dark-staining ectopic bone nearly occluding the medullary cavity **[2]**, and no visible periosteal formation or cortical bone continuity **[3]**.

4.3.1. LAB MEDIA: Figure 4A. *Video editor: Highlight panel a*.

4.3.2. LAB MEDIA: Figure 4A. *Video editor: Highlight the region with * and -> in panels i and ii beside 'a' at the top row*.

4.4. In the 3D printed PMMA group, newly formed bone filled the scaffold void, connected with cortical bone at the defect edges **[1]**, and was covered by continuous periosteal bone with no significant ectopic ossification in the marrow **[2]**.

4.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight panel b*.

4.4.2. LAB MEDIA: Figure 4A. *Video editor: Highlight panel b along with i and ii images beside b and focus on the regions marked with (●), and (▲)*

4.5. Immunofluorescence staining showed very few Piezo1-positive and LepR *(lep-R)*-

positive cells in regenerated bone in the Gelatin methacryloyl group **[1]**.

4.5.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the GelMA panel*.

4.6. In contrast, the 3D printed PMMA group displayed abundant Piezo1-positive cells and LepR-positive cells in the newly formed bone at the defect site **[1]**.

4.6.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the 3DP-PMMA panel*

4.7. Quantitative analysis confirmed that both Piezo1-positive and LepR-positive cell counts were significantly higher in the PMMA group compared to the Gelatin methacryloyl group **[1]**.

4.7.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the two taller bars for the 3DP-PMMA group.*

- Anesthetized

Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetized
IPA: /əˈniːsθəˌtaɪzd/
Phonetic Spelling: uh·nees·thuh·tyzd

- Lateral recumbent

Pronunciation link: https://www.merriam-webster.com/medical/recumbent
IPA: /ˈlætərəl rɪˈkʌmbənt/
Phonetic Spelling: lat·uh·ruhl rih·kum·buhnt

- Femur

Pronunciation link: https://www.merriam-webster.com/dictionary/femur
IPA: /ˈfiːmər/
Phonetic Spelling: fee·mer

- Iodine tincture

Pronunciation link: https://www.merriam-webster.com/medical/iodine%20tincture
IPA: /ˈaɪəˌdaɪn ˈtɪŋktʃər/
Phonetic Spelling: eye·uh·dyne tingk·cher

- Ethanol

Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθəˌnɔːl/
Phonetic Spelling: eth·uh·nawl

- Fenestrated

Pronunciation link: https://www.merriam-webster.com/dictionary/fenestrated
IPA: /ˈfɛnəˌstreɪtɪd/
Phonetic Spelling: feh·nuh·stray·tid

- Asepsis

Pronunciation link: https://www.merriam-webster.com/medical/asepsis
IPA: /eɪˈsɛpsɪs/
Phonetic Spelling: ay·sep·suhs

- Longitudinal

Pronunciation link: https://www.merriam-webster.com/dictionary/longitudinal
IPA: /ˌlɑːndʒəˈtuːdɪnəl/
Phonetic Spelling: lon·juh·too·duh·nuhl

- Rectus femoris

Pronunciation link: https://www.merriam-webster.com/medical/rectus%20femoris
IPA: /ˈrɛktəs ˈfɛmərɪs/
Phonetic Spelling: rek·tuhs feh·muh·riss

- Vastus lateralis

Pronunciation link: https://www.merriam-webster.com/medical/vastus%20lateralis
IPA: /ˈvæstəs ˌlætəˈrælɪs/
Phonetic Spelling: vas·tuhs lat·uh·ral·iss

- Fascia

Pronunciation link: https://www.merriam-webster.com/dictionary/fascia
IPA: /ˈfæʃə/
Phonetic Spelling: fash·uh

- Periosteal

Pronunciation link: https://www.merriam-webster.com/medical/periosteal
IPA: /ˌpɛriˈɑːstiəl/
Phonetic Spelling: peh·ree·os·tee·uhl

- Diaphysis

Pronunciation link: https://www.merriam-webster.com/medical/diaphysis
IPA: /daɪˈæfɪsɪs/
Phonetic Spelling: dye·af·uh·suhs

- Diaphyseal

Pronunciation link: https://www.merriam-webster.com/medical/diaphyseal
IPA: /ˌdaɪəˈfɪziəl/
Phonetic Spelling: dye·uh·fiz·ee·uhl

- Polymethyl methacrylate

Pronunciation link: https://www.merriam-webster.com/medical/polymethyl%20methacrylate
IPA: /ˌpɑːliˈmɛθəl ˌmɛθˈækrɪˌleɪt/
Phonetic Spelling: pah·lee·meth·uhl meth·ak·ruh·layt

- Monofilament

Pronunciation link: https://www.merriam-webster.com/dictionary/monofilament
IPA: /ˌmɑːnoʊˈfɪləmənt/
Phonetic Spelling: mon·oh·fil·uh·muhnt

- Iodophor

Pronunciation link: https://www.merriam-webster.com/dictionary/iodophor
IPA: /ˈaɪoʊdəˌfɔːr/
Phonetic Spelling: eye·oh·duh·for

- Micro-computed tomography

Pronunciation link: https://www.merriam-webster.com/medical/computed%20tomography
IPA: /ˈmaɪkroʊ kəmˈpjuːtɪd təˈmɑːgrəfi/
Phonetic Spelling: my·kroh kuhm·pyoo·tid tuh·mah·gruh·fee

- Ectopic ossification

Pronunciation link: https://www.merriam-webster.com/medical/ectopic
IPA: /ɛkˈtɑːpɪk ˌɑːsɪfɪˈkeɪʃən/
Phonetic Spelling: ek·top·ik ah·suh·fuh·kay·shuhn

- Medullary

Pronunciation link: https://www.merriam-webster.com/medical/medullary
IPA: /ˈmɛdʒəˌlɛri/
Phonetic Spelling: mej·uh·lair·ee

- Hematoxylin

Pronunciation link: https://www.merriam-webster.com/medical/hematoxylin
IPA: /ˌhiːməˈtɑːksɪlɪn/
Phonetic Spelling: hee·muh·tok·suh·lin

- Eosin

Pronunciation link: https://www.merriam-webster.com/medical/eosin
IPA: /ˈiːoʊsɪn/
Phonetic Spelling: ee·oh·sin

- Immunofluorescence

Pronunciation link: https://www.merriam-webster.com/medical/immunofluorescence
IPA: /ɪˌmjuːnoʊflʊˈrɛsəns/
Phonetic Spelling: ih·myoo·noh·floor·eh·suhns

- Piezo1

Pronunciation link: No confirmed link found
IPA: /ˈpiːeɪzoʊ wʌn/
Phonetic Spelling: pee·ay·zoh wuhn

- LepR

Pronunciation link: No confirmed link found
IPA: /ˈlɛp ɑːr/
Phonetic Spelling: lep ar